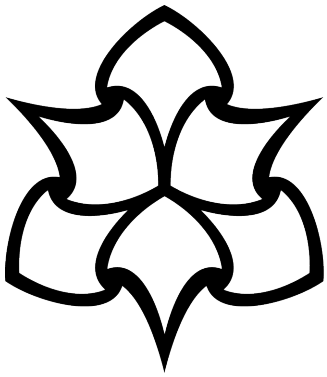

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Muscle Growth, Repair and Preservation: A Mechanistic Approach

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SUMMARY

Resistance exercise, amino acid ingestion and an anabolic hormone environment all have the capacity to elevate muscle protein synthesis (MPS), while a catabolic hormone environment, such as elevated pro-inflammatory cytokines as seen during disuse, aging, and conditions such as cancer and AIDS, can cause an increase in muscle protein degradation (MPD). When the rate of MPS exceeds that of MPD there is a positive net protein balance (NPB) and over a prolonged period of time this results in accretion of contractile material and muscle growth, or hypertrophy. In contrast, when NPB is chronically negative, muscle atrophy occurs, i.e. muscle size decreases. Various signaling pathways within the muscle fiber appear to play a crucial role in the adaptive processes, and understanding how these pathways can be modulated will help the design of therapies to prevent or reverse muscle atrophy in a host of muscle wasting conditions.

Key words: skeletal muscle – protein synthesis – hypertrophy – atrophy – IGF-I – mTOR – cytokine – TNF-α – interleukin – myostatin

INTRODUCTION

Skeletal muscle comprises numerous bundles of long, thin, multinucleated cells called muscle fibers, each containing a multitude of myofibrils. Each myofibril is composed of myofilaments (comprising the contractile proteins, actin and myosin) and a variety of structural proteins, all arranged in a regular configuration throughout the length of the myofibril, so as to form a series of contractile components, or sarcomeres. The maximum force that can be generated by a muscle fiber is proportional to the number of sarcomeres arranged in parallel, or fiber cross-sectional area (CSA), and ultimately the CSA of the whole muscle (Jones, Rutherford, Parker, 1989). Therefore, there is a strong relationship between whole muscle CSA and maximum isometric force measured *in vivo* (Bamman, Newcomer, Larson-Meyer, Weinsier, Hunter, 2000; Fukunaga et al., 2001; Kanehisa, Ikegawa, Fukunaga, 1994).

Based on the relationship between muscle size and force generating capacity (Erskine, Fletcher, Folland, 2014), it is not surprising that an increase in muscle size following resistance training (RT) is accompanied by an increase in maximal muscle force (Erskine, Jones, Williams, Stewart, Degens, 2010b; Jones, Rutherford, 1987; Narici et al., 1996). Not only can this enhance the athletic performance of an individual but it can also reduce the elevated risk of falling and bone fracture in older people that is among other factors attributable to sarcopenia (the age-related loss of muscle mass). The question thus arises as to the mechanisms underlying overload-induced muscle hypertrophy.

A multitude of signaling molecules within the muscle fiber are thought to play an integral role in stimulating muscle protein synthesis (MPS) and degradation (MPD). If

there is a positive net protein balance (NPB), i.e. when the rate of MPS exceeds that of MPD, the amount of contractile material will increase, enabling the muscle to hypertrophy and generate more force. Conversely, when NPB is negative, the muscle will decrease in size, or atrophy, and become weaker. This chapter will explore the specific signaling pathways involved in MPS and MPD, which help to explain how skeletal muscle adapts to overload, disuse, ageing and muscle-wasting diseases. Furthermore, strategies used to preserve or maintain muscle mass during periods of disuse and wasting, such as RT and nutritional interventions, will be discussed.

In addition to the mechanisms underlying muscle growth and atrophy, there is still more to be learned about the systems associated with repair following exercise-induced muscle damage. Several studies have reported that disruption of the cytoskeletal structure of muscle fibers is accompanied by impairment of muscular function following damage-inducing exercise (Baumert, Lake, Stewart, Drust, Erskine, 2016; Friden, Sjostrom, Ekblom, 1983; Lieber, Shah, Friden, 2002). As well as structural damage to the sarcomere, eccentric exercise can cause raised intracellular calcium ion (Ca^{2+}) levels (Belcastro, Albisser, Littlejohn, 1996), decreased muscle force production (Clarkson, Sayers, 1999; Friden et al., 1983), an increase in serum levels of muscle-specific proteins (Ebbeling, Clarkson, 1989), an increase in muscle specific inflammation (MacIntyre, Reid, McKenzie, 1995), an increase in proteolytic enzyme activity (Evans, Cannon, 1991), and a delayed-onset muscle soreness (MacIntyre et al., 1995). The ultimate repair of the muscle requires the activation of satellite cells and in this chapter we will consider the various MPD systems and the role of satellite cells thought to play a major role in muscle damage and repair following exercise.

MUSCLE GROWTH

While prenatal muscle growth is largely the result of muscle fiber formation, postnatal maturational muscle growth and that in response to RT is almost entirely attributable to fiber hypertrophy. Prenatal myogenesis, i.e. the formation of muscle fibers during embryonic development, involves the proliferation, migration, differentiation and fusion of muscle precursor cells to form post-mitotic multinucleated myotubes. Postnatal skeletal muscle growth is accompanied by an increase in the number of myonuclei per muscle fiber (Delhaas, van der Meer, Schaart, Degens, Drost, In Press) that requires the activation of muscle stem cells, or satellite cells (located at the basal lamina that surrounds the muscle fiber), which proliferate and fuse with existing muscle fibers (Jacquemin, Furling, Bigot, Butler-Browne, Mouly, 2004). Once fully mature, skeletal muscle growth, or hypertrophy, is dependent upon a positive NPB, i.e. MPS must be greater than MPD (Chesley, MacDougall, Tarnopolsky, Atkinson, Smith, 1992; Phillips, Tipton, Aarsland, Wolf, Wolfe, 1997), a process that is driven by an increase in the rate of MPS (Kumar, Atherton, Smith, Rennie, 2009). This leads to an accretion of myofibrillar proteins and an increase in muscle fiber CSA, which in turn leads to an increase in the overall CSA of the muscle, thus enabling more force to be produced. Resistance exercise, i.e. overloading the muscle, has been shown to increase MPS (Chesley et al., 1992; Phillips et al., 1997), and chronic resistance exercise, i.e. RT performed over many weeks, is a potent stimulus for skeletal muscle hypertrophy and strength gains (Erskine et al., 2010b; Jones et al., 1987; Narici et al., 1996). However, exactly how overloading the muscle leads to a positive NPB and therefore an increase in muscle size has yet to be fully elucidated. It is thought that the process necessary for inducing muscle hypertrophy involves a myriad of molecules

within the muscle fiber that form signaling cascades, eventually culminating in increased MPS and/or decreased MPD. Here we will discuss how insulin-like growth factor-I (IGF-I), mechanosensors, and amino acids might activate these specific signaling pathways that lead to MPS and ultimately to muscle growth, or hypertrophy.

The role of IGF-I in muscle growth

IGF-I is produced by the liver and skeletal muscle and thus acts on muscle fibers in an endocrine and autocrine/paracrine manner (Goldspink, 1999; Stewart, Rotwein, 1996). This growth factor appears to play an integral role in activating a specific signaling pathway within the muscle fiber that stimulates MPS (Bodine et al., 2001; Rommel et al., 2001). The local production and release of IGF-I during muscle contraction (DeVol, Rotwein, Sadow, Novakofski, Bechtel, 1990) activates this signaling cascade by binding to its receptor, located in the sarcolemma. This causes autophosphorylation of the insulin receptor substrate (IRS1) and subsequent phosphorylation of down-stream molecules within this signaling pathway, which includes phosphatidylinositol-3 kinase (PI3K), protein kinase-B (PKB or Akt), the mammalian target of rapamycin complex 1 (mTORC1), 70-kDa ribosomal S6 protein kinase ($p70^{S6K}$), and eukaryotic initiation factor 4E binding protein (4E-BP) (Fig. 1). In fact, $p70^{S6K}$ activation is related to gains in skeletal muscle mass following RT, both in rats (Baar, Esser, 1999) and humans (Terzis et al., 2008), with the increase occurring mainly in type II fibers (Koopman, Zorenc, Gransier, Cameron-Smith, van Loon, 2006). Together, these studies implicate mTORC1 and $p70^{S6K}$ as principal downstream mediators of IGF-I stimulation of skeletal muscle growth.

[FIGURE 1 NEAR HERE]

There is evidence that IGF-I produced in skeletal muscle is more important for developmental and exercise-induced muscle growth than IGF-I produced by the liver. This is indicated by the greater muscle mass in transgenic mice over-expressing IGF-I in skeletal muscle compared to wild-type mice (Coleman et al., 1995; Musaro et al., 2001) despite normal serum IGF-I levels (Coleman et al., 1995). Furthermore, low systemic IGF-I levels in liver-specific IGF-I knockout mice does not affect muscle size (Ohlsson et al., 2009; Yakar et al., 1999). Also in young adult men, elevated levels of circulating IGF-I do not influence MPS following an acute bout of resistance exercise (West et al., 2009) or muscle hypertrophy in response to RT (West et al., 2010). Although in rat skeletal muscle, local IGF-I gene expression increases proportionately to the progressive increase in external load (DeVol et al., 1990), it is equivocal whether this occurs in human muscle (Bamman et al., 2001; Bickel et al., 2005; Bickel, Slade, Haddad, Adams, Dudley, 2003; Hameed, Orrell, Cobbold, Goldspink, Harridge, 2003; Petrella, Kim, Cross, Kosek, Bamman, 2006; Psilander, Damsgaard, Pilegaard, 2003). Some of this controversy might be explained by the elevated expression of two isoforms of the *Igf1* gene in animal skeletal muscle in response to mechanical stimulation (McKoy et al., 1999; Yang, Alnaqeeb, Simpson, Goldspink, 1996). Thus, at least two IGF-I isoforms exist: (i) IGF-IEa, which is similar to the hepatic endocrine isoform, and (ii) the less abundant IGF-IEb (in rats) or IGF-IEc (in humans), otherwise known as mechanical growth factor (MGF). However, it is not always clear which IGF-I isoform has been measured in the muscle (Bamman et al., 2001). Furthermore, the age of the participants also influences the findings, with MGF increasing in young but not old people following an exercise bout. Interestingly, IGF-IEa does not appear to change in young or older people

following resistance exercise (Hameed et al., 2003), despite its apparent hypertrophic effect (Musaro et al., 2001).

Skeletal muscle-derived IGF-I does not appear to be the only regulator of adult muscle mass and function, as unloading induces skeletal muscle atrophy in mice whose muscles over-express IGF-I (Criswell et al., 1998), and overload induces hypertrophy even in transgenic mice, which express a dominant negative IGF-I receptor in skeletal muscle (Spangenburg, Le Roith, Ward, Bodine, 2008). Also in older people, enhanced muscle strength can be attained following RT without a significant change in muscle IGF-I gene expression (Taaffe, Jin, Vu, Hoffman, Marcus, 1996). Thus, it appears that for the development of hypertrophy in adult muscles, loading is more important than alterations in local and systemic IGF-I levels. This fits the notion that activation of mTORC1 and $p70^{S6K}$ can also occur independently of PI3K activation following muscle overload (Hornberger, Sukhija, Wang, Chien, 2007).

The role of mechanosensors in muscle growth

The process that couples the mechanical forces during a muscle contraction with cell signaling and ultimately protein synthesis is called mechanotransduction. It has been shown in rats that passive stretch induces an increase in the expression of myogenic regulatory factors (Kamikawa, Ikeda, Harada, Ohwatashi, Yoshida, 2013) that may well underlie the muscle fiber atrophy seen after passive stretching (Coutinho, DeLuca, Salvini, Vidal, 2006). These responses are probably at least partly mediated by stretch-activated channels (SACs), which are calcium (Ca^{2+}) and sodium permeable channels that increase their open probability (the fraction of time spent in

the open state) in response to mechanical loading of the sarcolemma (Franco, Lansman, 1990a; Franco, Lansman, 1990b; Guharay, Sachs, 1984). It has been proposed that SACs function as mechanosensors by allowing an influx of Ca^{2+} into the muscle fiber (Yeung et al., 2005) following mechanical changes in the sarcolemma, which activate mTORC1 (Gulati et al., 2008), leading to an increase in MPS (Kameyama, Etlinger, 1979). Correspondingly, inhibition of SACs by streptomycin reduces skeletal muscle hypertrophy in response to mechanical overload (Butterfield, Best, 2009; Spangenburg, McBride, 2006) via attenuation of mTORC1 and $p70^{S6K}$ activation (Spangenburg et al., 2006).

Other mechanosensors might exist in the form of costameres, intra-sarcolemmal protein complexes that are circumferentially aligned along the length of the muscle fiber (Pardo, Siliciano, Craig, 1983). Costameres mechanically link peripheral myofibrils via the Z-disks to the sarcolemma (Fig. 2), thus maintaining the integrity of the muscle fiber during contraction and relaxation (Pardo et al., 1983). An individual costamere contains many proteins arranged in a complex structure (Ervasti, 2003; Patel, Lieber, 1997), which comprises two different laminin receptors, a dystrophin/glycoprotein complex and an integrin-associated complex, which are localised in the sarcolemma and bound to intra and extra-cellular structural proteins (Fig. 2). Thus, the force-producing contractile material is connected to the basal membrane and ultimately to adjacent muscle fibers (Morris, Fulton, 1994; Patel et al., 1997; Rybakova, Patel, Ervasti, 2000).

[FIGURE 2 NEAR HERE]

Costameres are receptive to mechanical, electrical and chemical stimuli (Ervasti, 2003). Indeed, mechanical tension is essential in regulating costameric protein expression, stability and organization, with talin and vinculin, for instance, being up-regulated in response to muscle contraction (Tidball, Spencer, Wehling, Lavergne, 1999). Regular contractions, as experienced during RT, increase the expression of costameric proteins, such as desmin (Woolstenhulme, Conlee, Drummond, Stites, Parcell, 2006), alpha-1-syntrophin and dystrophin (Kosek, Bamman, 2008) in humans, while focal adhesion kinase (FAK) and paxillin activity are increased in stretch-induced hypertrophied avian skeletal muscle (Fluck, Carson, Gordon, Ziemiecki, Booth, 1999). The forces exerted on both the intracellular contractile proteins and the basal membrane during periods of loading are required to cause binding of basal membrane laminin to the receptors on the α and β integrins and on the dystrophin/glycoprotein complex (Fluck, Ziemiecki, Billeter, Muntener, 2002). Interaction between integrins and the extra-cellular matrix causes rapid phosphorylation of FAK (Cary, Guan, 1999), which subsequently activates $p70^{S6K}$ independently of Akt (Durieux et al., 2009; Klossner, Durieux, Freyssenet, Flueck, 2009). This probably occurs via the phosphorylation and, thus, inactivation of tuberous sclerosis complex 2 (Gan, Yoo, Guan, 2006; Malik, Parsons, 1996), thus activating mTORC1 as shown in Fig. 1.

The role of amino acids in muscle growth

Both resistance exercise (Biolo, Maggi, Williams, Tipton, Wolfe, 1995; Phillips et al., 1997) and amino acid/protein ingestion (Tang, Moore, Kujbida, Tarnopolsky, Phillips, 2009; Yang et al., 2012b) stimulate MPS independently, while a combination of the two augments MPS even further (Biolo, Tipton, Klein, Wolfe, 1997; Tipton,

Ferrando, Phillips, Doyle, Wolfe, 1999). Both stimuli cause an increase in mTORC1 activation (Apro, Blomstrand, 2010; Moore, Atherton, Rennie, Tarnopolsky, Phillips, 2011) but it is unclear whether they stimulate MPS via different signaling pathways, or whether the combination of the two stimulates the same pathway more than either stimulant on its own. It is thought that amino acids cause Rag GTPases to interact with raptor (a regulatory protein associated with mTORC1), leading to the translocation of mTORC1 to the lysosomal membrane, where Rheb (a Ras GTPase) activates mTORC1 (Kim, Guan, 2009; Sancak et al., 2010; Sancak et al., 2008), as shown in Fig. 1. Of the essential amino acids (EAAs), the branched-chain amino acids (BCAAs: isoleucine, leucine, and valine), and particularly leucine, are the most potent stimulators of the mTORC1 signaling pathway (Anthony et al., 2000). Leucine supplementation stimulates muscle protein accretion in cultured cells (Haegens, Schols, van Essen, van Loon, Langen, 2012) and can also reduce MPD in healthy men (Nair, Schwartz, Welle, 1992), and it is possible that the action of leucine occurs via its metabolite, β-hydroxy-β-methylbutyrate (HMB) (Aversa et al., 2011; Pimentel et al., 2011).

The timing of amino acid ingestion appears crucial for an optimal anabolic response to a single bout of resistance exercise: ingesting amino acids immediately before an exercise bout promotes a greater increase in MPS compared to ingestion immediately after the bout (Tipton et al., 2001). This effect was attributed to an increased blood flow during exercise, and therefore an increased delivery of amino acids to the active muscle when they were ingested prior to exercise (Tipton et al., 2001). As well as the timing, the amount of protein ingested is integral in producing an optimal anabolic environment following resistance exercise (Moore et al., 2009; Witard et al., 2014;

Yang et al., 2012a). For example, the MPS dose response to ingested protein after a single bout of resistance exercise in healthy young men is saturated at 20 g protein, and any additional ingested protein is simply oxidized (Moore et al., 2009; Witard et al., 2014). This suggests that if the rate of protein ingestion after resistance exercise exceeds the rate at which it can be incorporated into the muscle, the excess protein is not used for MPS. This dose-response relationship seems to be altered with age, as in older men increased rates of MPS were found when participants ingested 40 g protein following a resistance exercise bout (Yang et al., 2012a). Therefore, older muscle appears to be less sensitive to amino acids, which has been termed 'anabolic resistance', something that may be attributable to impaired ribosome genesis in older muscle (Chaillou, 2017). Finally, the type and quality of the ingested protein appears to be important when it comes to MPS (Tang et al., 2009; Yang et al., 2012b). Following a single bout of resistance exercise and the ingestion of whey, soy or casein, each containing 10 g EAA, larger increases in blood EAA, BCAA, and leucine concentrations were found following the ingestion of whey compared to either soy or casein (Tang et al., 2009), suggesting a greater availability of these amino acids for protein synthesis following whey protein ingestion. This may be a reflection of the different rate of protein digestion and absorption of amino acids between the protein types (Boirie et al., 1997; Dangin et al., 2001; Dangin et al., 2003) and explain why MPS was greater following ingestion of whey compared to casein, both at rest and after exercise (Tang et al., 2009). In older muscle, the rate of MPS appears to be greater with whey than soy protein ingestion following resistance exercise (Yang et al., 2012b), which could be due to the ~28% greater leucine content in whey versus soy protein (Drummond, Rasmussen, 2008) as well as differences in digestion and absorption rate.

There is therefore striking evidence to support the acute effects of amino acid ingestion and resistance exercise on MPS via their independent and complementary effects on mTORC1 activation. It is also well known that repeated bouts of resistance exercise over a prolonged period of time, i.e. a RT program, leads to gains in both muscle size and strength (Erskine et al., 2010b; Jones et al., 1987; Narici et al., 1996). Therefore, the amplification of the anabolic environment within the muscle seen with the combination of both amino acid/protein ingestion and resistance exercise (Biolo et al., 1997; Tipton et al., 1999) suggests that RT with protein supplementation should confer greater gains in skeletal muscle size and strength than RT alone. However, the evidence for protein supplementation enhancing the increases in muscle size and strength following longer term RT programs in young (Erskine, Fletcher, Hanson, Folland, 2012; Hartman et al., 2007) and older (Candow et al., 2008; Verdijk et al., 2009b) individuals is equivocal. The controversy surrounding the longer-term RT studies could be due to methodological differences/limitations between studies. For example, considerable inter-individual variability exists in the response to RT (Erskine, Jones, Williams, Stewart, Degens, 2010a; Hubal et al., 2005) and yet many studies have used small sample sizes (Godard, Williamson, Trappe, 2002; Hulmi et al., 2009; Willoughby, Stout, Wilborn, 2007) that may have limited the statistical power required to detect an influence of protein supplementation. Different measures of muscle hypertrophy may also compound this discrepancy. For example, some studies have determined muscle thickness using ultrasonography (Candow et al., 2008; Vieillevoye, Poortmans, Duchateau, Carpentier, 2010) or whole body fat-free mass assessed via dual-energy X-ray absorptiometry (Hartman et al., 2007), while others have used magnetic resonance imaging to provide a more precise assessment of

muscle size, but still found no effect of protein supplementation on muscle hypertrophy following RT (Coburn et al., 2006; Erskine et al., 2012; Holm et al., 2008; Hulmi et al., 2009). There are, however, circumstances where protein supplementation may have a beneficial effect on muscle hypertrophy and strength gains. For example, whole body RT (incorporating multiple muscle groups rather than an individual muscle) could create a requirement for an increase in exogenous protein (due to a greater absolute MPD) that might not be satisfied by habitual protein intake alone. This might be particularly beneficial in the early phase of a RT program when MPS and MPD are likely to be higher than towards the end (Hartman, Moore, Phillips, 2006; Phillips, Tipton, Ferrando, Wolfe, 1999). In addition, older individuals need to ingest at least twice as much protein (40 g) to maximally stimulate MPS (Moore et al., 2015; Yang et al., 2012a) compared to the 20 g dose in younger people (Moore et al., 2009; Witard et al., 2014), which may reflect an 'anabolic resistance' in old age. Therefore, previous studies that have not shown a beneficial effect of protein supplementation on muscle size and strength gains in older people may have been administering suboptimal amounts and types of protein per RT session. In frail elderly individuals, whose protein requirements are probably higher than in old, healthy people, more recent evidence supports the combination of 60 g/day milk protein supplementation and resistance exercise (twice weekly for six months) in increasing total leg lean mass composition over resistance training alone (Tieland et al., 2012).

MUSCLE ATROPHY

Skeletal muscle atrophy is known to occur in response to disuse and numerous chronic conditions, such as cancer, AIDS, and senile sarcopenia (the age-related loss of muscle mass). Here we will focus on the mechanisms underlying sarcopenia, which

is the major cause of muscle weakness in older individuals (Evans, 1995). Although the causes of sarcopenia are not fully understood, disuse, chronic systemic inflammation and neuropathic changes leading to motoneuron death are thought to play an integral role (Degens, 2010). Motoneuron death results in denervation of muscle fibers, and ultimately the loss of muscle fibers (hypoplasia). Selective atrophy of type II fibers (Lexell, Taylor, Sjostrom, 1988) and a decrease in the proportion of type II fibers (Jakobsson, Borg, Edstrom, Grimby, 1988; Larsson, 1983; Larsson, Sjodin, Karlsson, 1978) are thought to be caused by denervation accompanied by reinnervation of these fibers by axonal sprouting from adjacent slow-twitch motor units (Brooks, Faulkner, 1994; Faulkner, Larkin, Claflin, Brooks, 2007).

Chronic low-grade inflammation and sarcopenia

Physiological aging is associated with chronic low-grade inflammation, a condition that has been termed ‘inflammaging’ (Franceschi et al., 2000). Inflammaging is characterized by elevated serum levels of pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6 and tumor necrosis factor-α (TNF-α), as well as acute phase proteins such as C-reactive protein, or CRP (Bartlett et al., 2012; Erskine et al., 2017; Franceschi et al., 2000), and increased circulating levels are associated with lower muscle mass and weakness in old age (Erskine et al., 2017; Visser et al., 2002). Furthermore, the levels of cytokines that counteract the inflammatory state, such as IL-10, are reduced with age (Bartlett et al., 2012; Lio et al., 2002). The pro-inflammatory cytokines, IL-1, IL-6 and TNF-α are produced by both skeletal muscle fibers and adipose cells, and are therefore also members of the adipokine family. As we age, we accumulate more adipose tissue, which is deposited in the subcutaneous, visceral and intramuscular regions, and it is particularly the visceral fat that appears to

contribute to the inflammatory environment (Pedersen, 2009).

TNF-α induces the production of reactive oxygen species (ROS), altering vascular permeability, which leads to leukocyte infiltration of the muscle fiber (Evans et al., 1991) and further ROS generation by the leucocytes. This release of ROS also activates NF-*κ*B via degradation of I-*κ*B (Fig. 1), which results in the increased expression of key enzymes of the ubiquitin-proteasome MPD system (Li, Schwartz, Waddell, Holloway, Reid, 1998). In addition, TNF-α interferes with satellite cell differentiation and therefore muscle growth and regeneration in old age by reducing the expression of myogenic regulatory factors (MRFs) (Degens, 2010). The MRFs are a family of muscle-specific transcription factors (MyoD, myogenin, MRF4 and myf-5) that regulate the transition from proliferation to differentiation of the satellite cell (Langen et al., 2004; Szalay, Razga, Duda, 1997). The TNF-α-induced activation of NF-*κ*B results in a loss of MyoD mRNA (Guttridge, Mayo, Madrid, Wang, Baldwin, 2000) and, via activation of the ubiquitin–proteasome pathway (UPP) (Reid, Li, 2001; Saini, Al-Shanti, Faulkner, Stewart, 2008), breakdown of MyoD and myogenin. Thus, part of the attenuated hypertrophic response in elderly compared to younger muscle (Welle, Totterman, Thornton, 1996) could be due to a decrease in MRFs, as seen in overload-induced hypertrophy in older rats (Alway, Degens, Krishnamurthy, Smith, 2002a). TNF-α also stimulates the release of proteolytic enzymes, such as lysozymes (e.g. cathepsin-B) from neutrophils (Farges et al., 2002), which are thought to contribute to the MPD process (Friden, Kjorell, Thornell, 1984; Kasperek, Snider, 1985), but the main action of the pro-inflammatory cytokines in muscle atrophy is thought to occur via the UPP.

It has been suggested that the UPP cannot breakdown intact sarcomeres, so additional mechanisms are proposed to be involved (Solomon, Baracos, Sarraf, Goldberg, 1998). For example, the activation of caspase-3 is thought to lead to cleavage of the myofilaments, actin and myosin, which are then degraded by the UPP (Du et al., 2004; Lee et al., 2004; Ottenheijm et al., 2006). The UPP involves numerous enzymes, or ligases, that regulate the ubiquitination (the coupling of ubiquitin to protein substrates), so that the 'tagged' protein fragments can be identified by the proteasome for the final step of MPD (DeMartino, Ordway, 1998). These ubiquitin ligases may also degrade MyoD and inhibit subsequent satellite cell activation and differentiation (see above), thus exacerbating the effects of MPD on muscle size by impairing satellite cell associated muscle growth. Expression of two genes that encode the E3 ubiquitin ligases, muscle-specific atrophy F box (MAFbx, also known as Atrogin-1) and muscle RING Finger 1 (MuRF1), has been shown to increase during different types of muscle atrophy (Gomes, Lecker, Jagoe, Navon, Goldberg, 2001; Lecker et al., 2004). The structure and function of the UPP will be discussed in more detail, below, with regard to muscle damage and repair following exercise.

The role of myostatin in muscle atrophy

Myostatin, otherwise known as growth differentiation factor-8 (GDF-8), is part of the transforming growth factor β superfamily and is produced in skeletal muscle. The role of myostatin as a negative regulator of muscle mass has been demonstrated by knocking out the *gdf-8* gene in mice, which leads to a 2-3 fold increase in skeletal muscle mass (McPherron, Lawler, Lee, 1997). Conversely, administering myostatin to wild-type mice induces substantial muscle wasting (Zimmers et al., 2002). Further examples of myostatin's regulatory effect can be seen in bovine (McPherron, Lee,

1997) and human (Schuelke et al., 2004) cases, where mutation of the *gdf -8* gene leads to a reduction in myostatin production and considerably enlarged skeletal muscles.

Once bound to the activin IIB receptor (ActRIIB), a signaling cascade is activated that leads to MPD. Key signaling proteins in this pathway include SMAD 2 and 3 (Sartori et al., 2009), which form a complex with SMAD 4 that then translocates to the nucleus where it targets genes encoding MRFs (Rodino-Klapac et al., 2009), and inhibits differentiation via the reduction of MyoD expression (Langley et al., 2002) (Fig. 1). In addition, myostatin reduces Akt/ mTORC1/$p70^{S6K}$ signaling (Amirouche et al., 2009; Trendelenburg et al., 2009) (Fig. 1) and is associated with smaller myotube size (Trendelenburg et al., 2009). Accordingly, the inhibition of myostatin in mature mice leads to increased activation of $p70^{S6K}$, ribosomal protein S6 and skeletal muscle MPS (Welle, Burgess, Mehta, 2009). Myostatin appears to not only inhibit satellite cell differentiation and MPS, but also to induce the expression of atrogenes (genes associated with muscle atrophy) via activation of the p38 mitogen-activated protein (MAP) kinase, Erk1/2, Wnt and c-Jun N-terminal kinase (JNK) signaling pathways (Huang et al., 2007; Philip, Lu, Gao, 2005; Yang et al., 2006) (Fig. 1). This is in accord with the observation that myostatin induces cachexia by activating the UPP, i.e. phosphorylating the ubiquitin E3 ligases MAFbx and MuRF1, via FOXO1 activation rather than via the NF-κB pathway (McFarlane et al., 2006). However, myostatin-induced atrophy persists despite inhibiting the expression of the two E3 ligases (Trendelenburg et al., 2009). Therefore, it is likely that myostatin negatively regulates muscle growth via multiple pathways (Fig. 1). A lower expression of myostatin may therefore help to maintain muscle mass at old age, a situation reflected

by the attenuated loss of muscle mass and regenerative capacity in old myostatin-null mice compared to age-matched wild-type mice (Siriett et al., 2006).

Combating sarcopenia

RT has been shown to increase muscle size and strength in old (Reeves, Narici, Maganaris, 2004), very old (Harridge, Kryger, Stensgaard, 1999) and frail (Fiatarone et al., 1994) individuals. This beneficial effect of RT is attributable to an increase in MPS in the atrophied muscle (Kimball, O'Malley, Anthony, Crozier, Jefferson, 2004) and a reduction in MPD as a result of a reduced MAFbx and MuRF-1 gene expression (Jones et al., 2004; Mascher et al., 2008). A reduction in atrogene expression can be realized by the ability of phosphorylated Akt to block FoxO1, which would suppress the transcription of MuRF1 and MAFbx (Sandri et al., 2004), as shown in Fig. 1. In addition to the Akt/FoxO-1 pathway, mTORC1 also blocks MuRF1 and MAFbx transcription (Sandri et al., 2004). Therefore, while a degree of MPD is required for muscle remodeling, RT appears to reverse atrophy via the inhibiting effect of Akt/mTORC1 on MPD and the positive effect of mTORC1 activation on MPS (Apro et al., 2010; Moore et al., 2011), thus resulting in net protein synthesis (albeit to a lesser extent in elderly compared to younger muscle).

The apparent ‘anabolic resistance’ to RT in older compared to young muscle (Kimball et al., 2004; Welle et al., 1996) may not be due to an age-related reduction in the mechanosensitivity of the mTORC1 signaling pathway (Hornberger, Mateja, Chin, Andrews, Esser, 2005), although others do see a reduction in the translational signaling during overload (Thomson, Gordon, 2006). It could therefore be that the

rates of transcription and translation are reduced during overload, which may be a consequence of impaired ribosome biogenesis in older muscle (Chaillou, 2017).

Another factor that might be considered is the proposed requirement of satellite cell recruitment for the development of hypertrophy. An impaired satellite cell recruitment would then result in impaired hypertrophy and part of the problem might be a decline in satellite cell number (Shefer, Van de Mark, Richardson, Yablonka-Reuveni, 2006), particularly in type II muscle fibers of older people (Verdijk et al., 2007). Yet, paradoxically, older muscle appears to have an increased regenerative drive and protein synthesis that is more pronounced the more severe the sarcopenia. For instance, muscle mass is lower despite higher $p70^{S6K}$ activation and MPS in old compared to young adult rats (Kimball et al., 2004). IGF-I appears to play a key role in the activation and proliferation of satellite cells (Scime, Rudnicki, 2006), while differentiation is regulated by MRFs (Langen et al., 2004; Szalay et al., 1997). However, despite an increased regenerative drive as reflected by elevated IGF-I expression (Edstrom, Ulfhake, 2005) and MRF mRNA expression (Alway, Degens, Lowe, Krishnamurthy, 2002b) in old rat muscle, MRF protein levels are reduced (Alway et al., 2002b). This reduction might be caused by a concomitant increase in Id protein expression (Alway et al., 2002b), which inhibits MRF expression and DNA binding capacity. The result is that during an hypertrophic stimulus, satellite cell activation and proliferation can occur in older rat skeletal muscle but with limited differentiation (Edstrom et al., 2005). In elderly human skeletal muscle, however, the capacity for satellite cells to proliferate and differentiate in response to RT does not appear to be diminished (Mackey et al., 2007; Verdijk et al., 2009a). It should be noted, however, that the relative age in the human studies was less than that in the rat

studies and it may be that beyond a given age in humans, the differentiation of satellite cells may also be diminished, particularly when associated with chronic low-grade systemic inflammation.

Although satellite cells are generally considered to play an important role in muscle hypertrophy, the conditional ablation of satellite cells by tamoxifen in Pax7-DTA mice did not attenuate the development of 100% hypertrophy induced by overload (McCarthy et al., 2011). It may well be that the microcirculation is crucial for the development of hypertrophy as it has been found that in genetically modified mice with muscle hypertrophy (due to myostatin knock out) and high-oxidative fibers (overexpression of oestrogen-related receptor gamma) the regenerative capacity was normal despite a lower satellite cell content, but higher capillary density (Omairi et al., 2016). Also in aged mice, the blunted hypertrophic response was associated not only associated with a lower satellite cell content (Ballak et al., 2015), but also with impaired angiogenesis (Ballak et al., 2016). These data suggest that impaired angiogenesis and/or reductions in the capillarization, that would result in an impaired delivery of substrates, of the muscle may underlie the impaired regenerative capacity and anabolic resistance in old age

Extra stimulation of the mTORC1 pathway may overcome the anabolic blunting. As discussed above, older people need to ingest more protein than younger individuals to stimulate maximal MPS (Moore et al., 2015; Moore et al., 2009; Volpi, Mittendorfer, Rasmussen, Wolfe, 2000; Yang et al., 2012a). The greater activation of the mTORC1 pathway when combining RT with protein/amino acid ingestion may inhibit MPD and augment MPS, thereby improving the hypertrophic response. The observation that

BCAA administration attenuates the loss of body mass in mice bearing a cachexia-inducing tumor (Eley, Russell, Tisdale, 2007) is promising and suggests that it may also enhance the hypertrophic response in this condition. Furthermore, HMB has been shown to attenuate the reduction in MPS in rodents following the administration of a cachectic stimulant (Aversa et al., 2011; Eley, Russell, Baxter, Mukerji, Tisdale, 2007).

In addition to RT and amino acid supplementation, various pharmaceutical therapies have been proposed to combat sarcopenia. Supplementation of the anabolic steroid, testosterone, augments muscle mass in older men, healthy hypogonadal men, older men with low testosterone levels, and men with chronic illness and low testosterone levels (Bhasin et al., 2006). It is thought that testosterone can reverse sarcopenia by suppressing skeletal muscle myostatin expression, while simultaneously stimulating the Akt pathway (Kovacheva, Hikim, Shen, Sinha, Sinha-Hikim, 2010) to increase MPS and decrease MPD. Furthermore, administration of a myostatin antagonist has led to satellite cell activation, increased MyoD protein expression, and greater muscle regeneration after injury in old murine skeletal muscle (Siriett et al., 2007).

MUSCLE DAMAGE AND REPAIR

In normal skeletal muscle, cytoskeletal proteins act as a framework that keeps the myofibrils aligned in a lateral position by connecting the Z-disks to one another and to the sarcolemma (Friden et al., 1984; Friden et al., 1983). Following eccentric exercise, Z-disk streaming (disturbance of Z-disk configuration) and misalignment of the myofibrils is a common characteristic (Friden et al., 1984). Eccentric contractions are defined as contractions where muscles lengthen as they exert force and generally

result in more muscle damage than concentric contractions (Clarkson, Hubal, 2002). It has been suggested that this is due to fewer motor units being recruited during eccentric exercise, leading to a smaller CSA of muscle being activated than during a concentric contraction at the same load (Enoka, 1996). It has also been demonstrated that the extent of muscle damage is due to strain (the change in length) rather than the amount of force generated by the muscle (Lieber, Friden, 1993; Lieber, Friden, 1999).

Eccentric exercise not only causes alterations in the cytoskeletal structure, but also increases in the activity of proteolytic enzymes (Arthur, Booker, Belcastro, 1999; Kasperek et al., 1985; Stupka, Tarnopolsky, Yardley, Phillips, 2001). The positive correlation between the proteolytic enzyme activity and a rise in serum concentrations of muscle-specific proteins, e.g. creatine kinase (CK), post exercise (Arthur et al., 1999; Kasperek et al., 1985; Stupka et al., 2001), suggests that the degree of activation of the proteolytic machinery is related to the degree of muscle damage. Therefore, it is feasible that the activity of these proteolytic enzymes may be required for the remodeling of skeletal muscle in response to exercise, where the regulated degradation of cellular proteins (Ordway, Neufer, Chin, DeMartino, 2000) may be a pre-requisite for subsequent adaptive repair and growth.

There are three main systems that contribute to the controlled MPD following muscle damage; 1) the release of calpain, a non-lysosomal, Ca^{2+}-dependent neutral protease that mediates the dismantling of myofibrils (Belcastro, Shewchuk, Raj, 1998), 2) the inflammatory response, which includes lysosomal proteolysis (Farges et al., 2002), and 3) the ATP-dependent UPP, which coordinates the demolition of protein fragments liberated by the aforementioned degradation systems (DeMartino et al.,

1998). Recent findings suggest that myostatin is also implicated in the MPD process following damaging muscle contractions (Ochi et al., 2010).

The calpain protein degradation system

Calpain is a multidomain protein composed of two subunits, a catalytic 80-kDa subunit and a regulatory 30-kDa subunit (Suzuki, Sorimachi, Yoshizawa, Kinbara, Ishiura, 1995). In skeletal muscle, three homologous isozymes of calpain with different Ca^{2+} sensitivities have been identified (DeMartino et al., 1998): μ-calpain (active at micromolar Ca^{2+} concentrations), m-calpain (active at millimolar Ca^{2+} concentrations), and n-calpain (requiring very high Ca^{2+} concentrations). It appears that, although the μ- and m-calpain 80-kDa subunits are quite different, both have similar binding domains; the proteolytic site of a cysteine proteinase, the calpastatin (an endogenous inhibitor of calpain activity) binding domain, and the Ca^{2+}-binding domain (Belcastro et al., 1996). The 30-kDa subunit is extremely hydrophobic, which may help to act as an anchor to the membrane proteins (Belcastro et al., 1996). While there is evidence to suggest calpain is localized and activated at or around the sarcolemma, thus targeting the membrane-associated proteins (Belcastro et al., 1998), others have demonstrated that calpain also targets Z-disk proteins, such as desmin and α-actinin (Goll, Dayton, Singh, Robson, 1991). The action of other proteolytic complexes, including lysosomal enzymes and the UPP, may have a part to play in MPD immediately after damaging eccentric muscle contractions, but as their activity does not peak until later in the muscle damage/repair process (Belcastro et al., 1998; Kasperek et al., 1985), it is more likely that calpain and/or mechanical stress is the initial effector of cytoskeletal protein breakdown.

Calpain is activated by raised intracellular [Ca^{2+}] (Belcastro et al., 1998). Initial mechanical damage to the sarcoplasmic reticulum (SR) and muscle plasma membrane caused by eccentric muscle contractions could lead to SR vacuolization and an increase in intracellular [Ca^{2+}] (Clarkson et al., 2002; Warren, Hayes, Lowe, Armstrong, 1993). The intracellular [Ca^{2+}] could rise further following an increased open probability of SACs as a consequence of increased strain on the skeletal muscle fibers during forced lengthening (Lieber et al., 1993). It is thought that the activation of calpain is pivotal in the breakdown of cytoskeletal proteins, including desmin and α-actinin, rather than mechanical stress applied to the "over-stretched" sarcomeres during eccentric contractions *per se* (Belcastro et al., 1998). The activity of calpain is not only dependent on the intracellular [Ca^{2+}] but also on the concentration of its inhibitor, calpastatin, and condition of degradable substrates, i.e. the ultrastructural proteins. To become fully active, calpain undergoes autolysis into its subunits. It is likely that the influx of excess Ca^{2+} into the muscle fiber (via the SACs, SR calcium channels and sarcolemmal lesions) binds to the specific domain on the 80-kDa calpain subunit, thereby inhibiting calpastatin. Once calpain is free of calpastatin, it may begin autolysis and/or bind to its substrate (with the help of Ca^{2+}) and begin the process of MPD (Belcastro et al., 1996). A positive relationship between calpain activity and neutrophil accumulation within skeletal muscle after exercise suggests that the calpain-degraded protein fragments act as chemoattractants, thus localizing leukocytes to the site of muscle damage (Belcastro et al., 1998; Raj, Booker, Belcastro, 1998).

The inflammatory response

Exercise-induced damage to muscle fibers elicits an inflammatory response that

results in movement of fluid, plasma proteins and leukocytes to the site of injury (Clarkson et al., 2002). Leukocytes have the ability to break down intracellular proteins with the aid of lysosomal enzymes (Friden et al., 1984), but exactly how the inflammatory response regulates MPD and muscle repair following eccentric exercise is not entirely clear. However, the purpose of the post-exercise-induced inflammatory response is to promote clearance of damaged muscle tissue and prepare the muscle for repair (MacIntyre et al., 1995), a process that is sub-classified into acute and secondary inflammation.

The acute phase response in skeletal muscle begins with the 'complement system' when fragments from the damaged fiber(s) serve as chemoattractants, luring leukocytes to the injured area (Belcastro et al., 1998; Evans et al., 1991). As a consequence there is an accumulation of neutrophils, the histological hallmark of acute inflammation (MacIntyre et al., 1995), in and around the site of injury, which peaks around 4 hours after exercise-induced damage has occurred (Evans et al., 1991). The accumulation of neutrophils has been reported to be more significant after eccentric than concentric exercise, and is most likely related to the degree of damage incurred (Evans et al., 1991). Pro-inflammatory cytokines, such as IL-1, IL-6, TNF-α, and acute phase proteins, e.g. CRP, act as mediators of inflammatory reactions. TNF-α induces the production of ROS, altering vascular permeability, which leads to leukocyte infiltration into the muscle fiber (Evans et al., 1991). TNF-α also stimulates the release of cytoxic factors from neutrophils, such as lysozymes and ROS (Friden et al., 1984; MacIntyre et al., 1995), which are responsible for at least a part of the MPD process following exercise-induced damage (Friden et al., 1984; Kasperek et al., 1985).

It may take up to seven days to see a significant infiltration of monocytes (precursors to macrophages) within the damaged muscle fiber (Evans et al., 1991), which carry out further phagocytic activity inside the muscle fiber. Furthermore, considerable increases in the quantity of lipofuscin granules (generally considered to be the indigestible residue of lysosomal degradation) in sore muscles three days after exercise suggests that lysozyme activity plays a major part in the secondary inflammatory process (Farges et al., 2002; Friden, 1984). The role of the inflammatory response in muscle regeneration is therefore thought to be the further breakdown of damaged muscle proteins via lysozymes, the engulfing of protein fragments by macrophages and the activation of the UPP by the pro-inflammatory cytokines released from the neutrophils. These cytokines simultaneously stimulate the proliferation of satellite cells, crucial for the regeneration of the damaged area (Chen, Jin, Li, 2007).

The ubiquitin-proteasome pathway

This pathway is recognized as the major non-lysosomal complex responsible for the degradation of cellular proteins. The UPP has received much attention due to its involvement in cellular processes, where protein degradation is a key regulatory or adaptive event (Attaix et al., 1998; Attaix, Combaret, Pouch, Taillandier, 2001; Ciechanover, 1994). There are two types of ubiquitin in human skeletal muscle: free and conjugated (Thompson, Scordilis, 1994). In its free state ubiquitin is a normal component of the non-stressed muscle fiber but it also forms complexes, or conjugations, with abnormal proteins and then returns to its free state (Fig. 3). The conjugation of ubiquitin with denatured proteins within the muscle fiber “tags” these

proteins for recognition by a non-lysosomal protease to be subsequently degraded in a process that requires ATP (Attaix et al., 1998). The UPP, therefore, consists of two major components that represent the system's functionally distinct parts. Ubiquitin is the element that covalently binds to the protein due to be broken down, while the 26S proteasome, a large protease complex, catalyses the degradation of the ubiquitin-tagged proteins (DeMartino et al., 1998).

[FIGURE 3 NEAR HERE]

The cellular proteins are selected for degradation by the attachment of multiple molecules of ubiquitin, or a polyubiquitin chain, which is built by repeated cycles of conjugation via the action of E1, E2, and E3 conjugating enzymes (Fig. 3). Ubiquitin is initially activated in the presence of ATP by the ubiquitin-activating enzyme, E1, which then transfers ubiquitin to E2, one of the ubiquitin-conjugating enzymes. E2 then binds the ubiquitin molecule to the protein substrate, which is selected for tagging by E3 (Attaix et al., 2001). The 26S proteasome is able to discriminate between ubiquitinated and non-ubiquitinated proteins, and rapidly degrades the polyubiquitinated proteins, deriving the energy for this process from ATP hydrolysis (Hershko, Ciechanover, 1998).

The 26S proteasome is composed of a 20S proteasome and two 19S (PA700) regulatory modules (Attaix et al., 2001; Ciechanover, 1994; DeMartino et al., 1998). The 20S proteasome is the proteolytic core, containing multiple catalytic sites. PA700 binds to each end of the proteasome cylinder and elicits ATPase activity in order to unfold and/or translocate the ubiquitinated proteins to the catalytic sites within the

20S proteasome (Fig. 3). PA700 is also thought to be responsible for disassembling the polyubiquitinated chain, a process requiring its isopeptidase activity.

The total amount of ubiquitin found in skeletal muscle is muscle fiber-type specific, with a greater abundance of ubiquitin found in type I fibers (Riley et al., 1992). Furthermore, a three to seven times higher density of conjugated ubiquitin was found at the Z-discs than anywhere else in the muscle fiber, which suggests that, like calpain, ubiquitin targets the cytoskeletal proteins of muscle fibers. One main difference between the two systems, however, is that calpain is activated a lot earlier than the ubiquitin-proteasome pathway (Feasson et al., 2002; Stupka et al., 2001). Furthermore, the action of the UPP may be prolonged post-exercise in order to increase the intracellular concentration of amino acids (Tipton, Wolfe, 1998), which would stimulate MPS via mTORC1 activation.

Repair following exercise-induced muscle damage

Following the orderly demolition of damaged/cleaved muscle proteins (via the aforementioned MPD systems) in response to eccentric contractions, the damaged muscle fibers need to undergo repair. The activation, proliferation and fusion of satellite cells with damaged muscle fibers, and the subsequent differentiation into myoblasts, are crucial for this repair process (McCarthy et al., 2011; Petrella et al., 2006). In fact, it has been shown that while the hypertrophic response maybe maintained in the absence of satellite cell recruitment, recovery from damage was severely impaired under these conditions (McCarthy et al., 2011).

As previously discussed, IGF-I and MRFs are integral in the activation and differentiation of satellite cells (Langen et al., 2004; Scime et al., 2006; Szalay et al., 1997), which probably explains why skeletal muscle IGF-I and MRF expression are increased following stretch-induced damage (Bickel et al., 2005; Petrella et al., 2006; Yang, Alnaqeeb, Simpson, Goldspink, 1997; Yang, Creer, Jemiolo, Trappe, 2005). To repair the muscle, satellite cells fuse with damaged fibers and differentiate into myonuclei, but may even form new fibers in the case of complete fiber necrosis. The orderly proliferation and subsequent differentiation is crucial for optimal repair. For the initial proliferation of satellite cells the inflammatory environment is beneficial, but this inflammation must be transient to allow the cells to differentiate (Pelosi et al., 2007). Therefore, it may be that chronic low-grade systemic inflammation, e.g. during ageing, may underlie the delay in muscle regeneration (Langen et al., 2006).

SUMMARY

Skeletal muscle is able to hypertrophy in response to a variety of anabolic stimuli, which include resistance exercise, amino acid ingestion, and an increase in IGF-I expression. All these stimuli are able to activate the mTORC1 signaling pathway, which stimulates MPS and inhibits MPD. When the rate of MPS exceeds MPD, there is a positive net protein balance (NPB) and an accretion of contractile material occurs, leading to muscle hypertrophy and an increase in strength. Inducing muscle hypertrophy can have beneficial effects on individuals suffering from cachectic conditions, such as cancer, AIDS, and sarcopenia, where muscle atrophy can have devastating effects on an individual’s quality of life. Muscle atrophy occurs when there is a negative NPB, i.e. when the rate of MPD is greater than MPS. There are a number of stimuli that have been associated with muscle atrophy, including

chronically elevated levels of pro-inflammatory cytokines (e.g. IL-1, IL-6 and TNF-α), a reduction in IGF-I and increased expression of myostatin. Furthermore, strenuous unaccustomed exercise can cause mechanical damage to the muscle, which activates MPD systems, including calpain, inflammation and the ubiquitin-proteasome protein degradation pathway. Damaged proteins within the muscle fiber are broken down, resulting in an increased intracellular amino acid concentration, which in turn activates mTORC1 and increases MPS, thus helping to repair the muscle. Elevated local IGF-I and MRF expression facilitates the repair process by activating satellite cells and enabling fusion with existing fibers. Many of the molecular signaling pathways associated with muscle hypertrophy, atrophy and repair have been identified. However, there is still much to be learned about these pathways, and understanding them may help us to prevent or reverse muscle atrophy associated with a host of muscle wasting conditions.

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FIGURE LEGENDS

Figure 1. The molecular signaling pathways associated with muscle hypertrophy and atrophy. The binding of IGF-I to its receptor (IGF-IR) causes autophosphorylation of insulin receptor substrate (IRS1). Phosphatidylinositol 3-kinase (PI3K) is a lipid kinase that phosphorylates phosphatidylinositol (4,5)-bisphosphate, producing phosphatidylinositol (3,4,5)-trisphosphate, which is a membrane-binding site for phosphoinositide-dependent protein kinase (PDK1). Upon translocation to the sarcolemma, AKT (or protein kinase B, PKB) is phosphorylated by PDK1. Once activated, AKT phosphorylates mammalian target of rapamycin complex 1 (mTORC1) directly and by phosphorylating and inactivating the tuberous sclerosis complexes 1 and 2 (TSC1/2), which otherwise inhibit mTORC1 activation. Following resistance exercise, an influx of calcium ions (Ca^{2+}) via stretch-activated channels (SACs) and the activation of FAK in the costamere can inactivate TSC1/2, thus activating mTORC1. Amino acids entering the muscle fiber cause RagGTPase-dependent translocation of mTORC1 to the lysosome, where it is activated by ras homologous protein enriched in brain (Rheb). mTORC1 subsequently activates 70KDa ribosomal S6 protein kinase ($p70^{S6K}$), and inhibits 4E-BP (also known as PHAS-1), which is a negative regulator of the eukaryotic translation initiation factor 4E (eIF-4E). Phosphorylated AKT also inhibits glycogen-synthase kinase 3β (GSK3β), a substrate of AKT that blocks protein translation initiated by the eIF-2B protein. All of these actions lead to increased protein synthesis. However, protein degradation can be induced by pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which activate NF-*κ*B via degradation of I-*κ*B, leading to increased transcription of the E3 ubiquitin-ligase, muscle RING-finger protein-1 (MuRF1). Another ligase, Atrogin1 (also known as MAFbx), is up-regulated by mitogen-activated protein kinase (MAPK) p38, while both ligases are up-regulated by forkhead box (FOXO) transcription factors. However, phosphorylated AKT blocks the transcriptional up-regulation of Atrogin1 and MuRF1 by inhibiting FOXO1, while phosphorylated mTORC1 inhibits the up-regulation of Atrogin1 directly. Myostatin also increases protein degradation and decreases protein synthesis by activating MAPKs and the SMAD complex, and by inhibiting PI3K. In addition, myostatin inhibits the myogenic program, thus resulting in a decrease of myoblast proliferation.

Figure 2. Schematic representation of (A) the location of costameres within a skeletal muscle fiber and (B) the proteins that constitute the costamere. Costameres are protein complexes circumferentially aligned along the length of the muscle fiber that connect peripheral myofibrils at the Z-disks to the sarcolemma and beyond to the extra-cellular matrix (ECM). The costamere comprises a dystrophin/glycoprotein complex and a focal adhesion complex (FAC), which includes the integrin-associated tyrosine kinase focal adhesion kinase (FAK). Fig. 2B modified from (Fluck et al., 2002) with permission.

Figure 3. Breakdown of the protein fragment via the ubiquitin proteasome pathway. Multiple ubiquitin (Ub) molecules form a polyubiquitin chain in a process involving the Ub-activating (E1), Ub-conjugating (E2), and Ub-ligating (E3) enzymes. The targeted protein fragment is then selected for degradation, or "tagged", via the covalent attachment of the polyubiquitin chain. The tagging enables the 19S (PA700) module to recognize the protein fragment, so that it can be further degraded by the 20S core into oligopeptides after it has been de-ubiquitinated and the Ub molecules released recycled. Adapted from (Milacic, Dou, 2009) with permission.

Figure 1

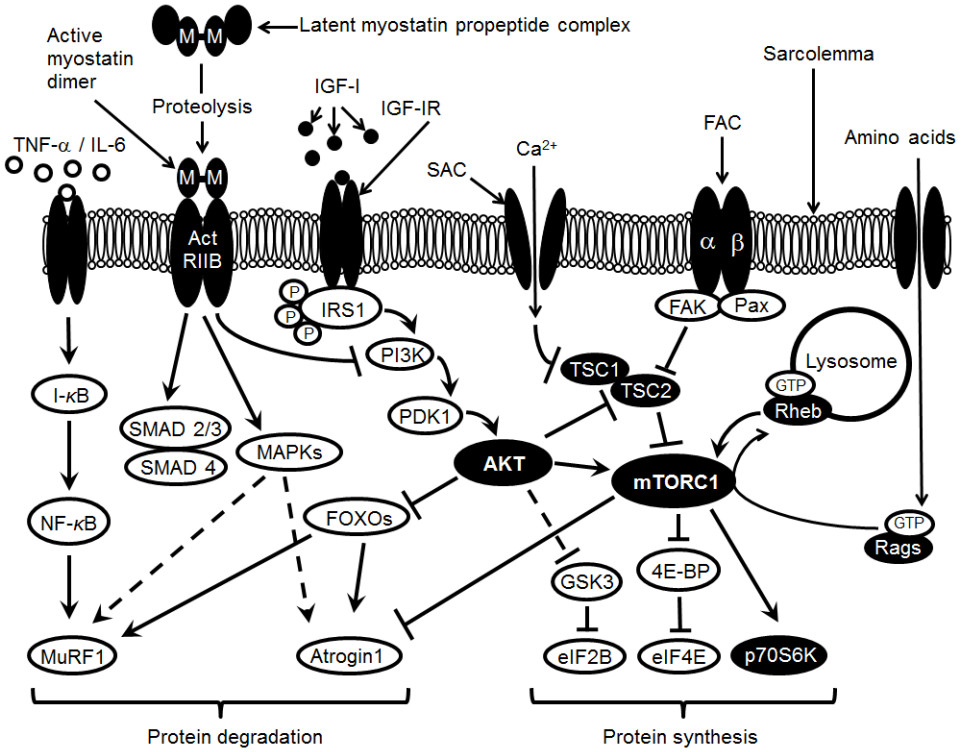
Active
myostatin
dimer
M-M
Latent myostatin propeptide complex
Proteolysis
IGF-I
IGF-IR
Sarcolemma
FAC
Amino acids
TNF-α / IL-6
Ca2+
SAC
M-M
Act
RIIB
α β
P
P
P
IRS1
PI3K
FAK
Pax
Lysosome
GTP
Rheb
TSC1
TSC2
I-κB
SMAD 2/3
SMAD 4
MAPKs
PDK1
AKT
mTORC1
NF-κB
FOXOs
GTP
Rags
GSK3
4E-BP
MuRF1
Atrogin1
eIF2B
eIF4E
p70S6K
Protein degradation
Protein synthesis

Figure 2A

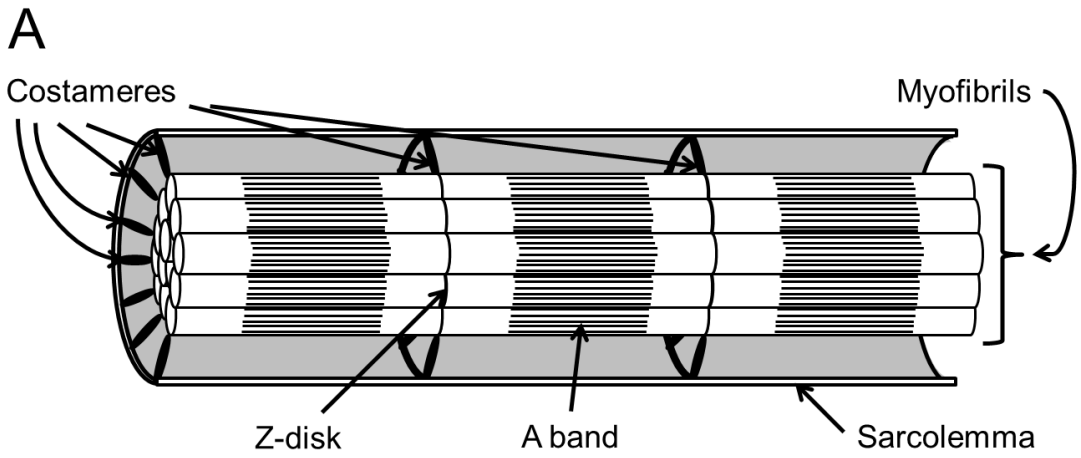

Figure 2B

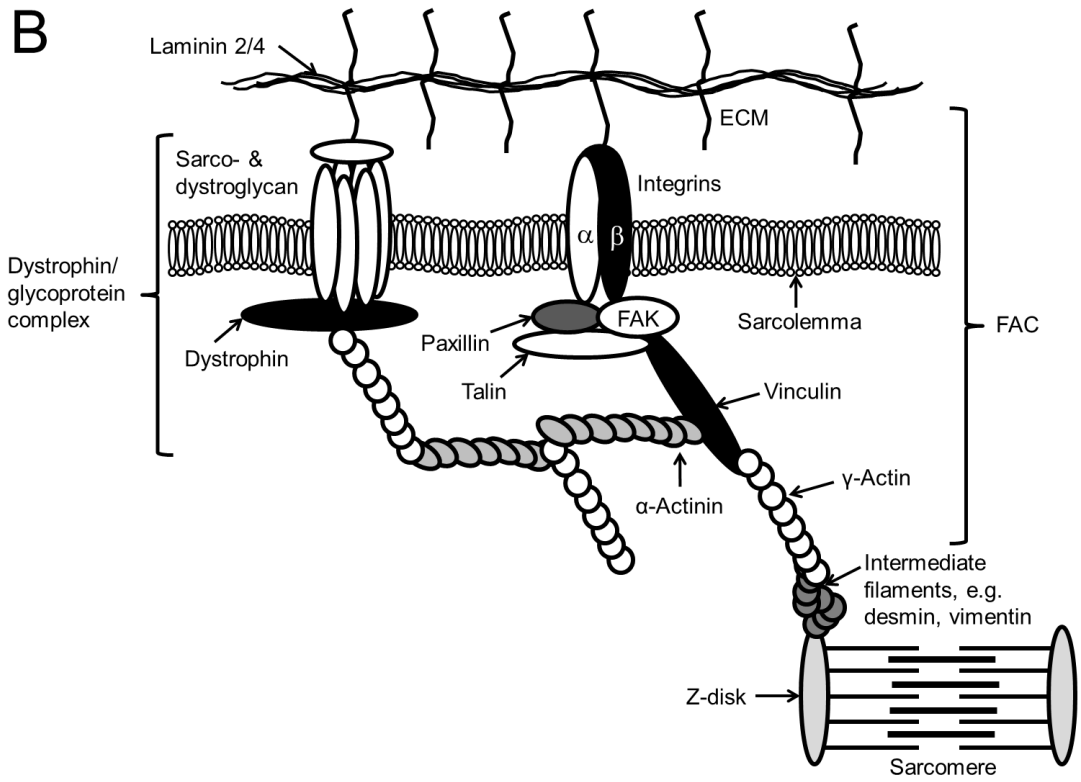

Figure 3